



## Tests for adaptive RAPD variation in population genetic structure of wild barley, *Hordeum spontaneum* Koch.

SERGEI VOLIS<sup>1,2\*</sup>, BAHTIYOR YAKUBOV<sup>3†</sup>, IRINA SHULGINA<sup>1</sup>, DAVID WARD<sup>2‡</sup>, VARDA ZUR<sup>1</sup> and SAMUEL MENDLINGER<sup>1</sup>

<sup>1</sup>The Institutes for Applied Research, Ben-Gurion University of the Negev, POB 653, Beer Sheva 84105, Israel

<sup>2</sup>The Mitrani Department for Desert Ecology, The Jacob Blaustein Institute for Desert Research, Ben-Gurion University of the Negev, Sede Boqer 84990, Israel

<sup>3</sup>The Institute of Plant Genetics and Experimental Biology, POB 97, Tashkent 700000, Uzbekistan

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We examined the adaptive importance of RAPD variation in the population genetic structure of wild barley, *Hordeum spontaneum*. The test involved (1) a nested sampling design with four population groups representing four distinct environments; and (2) a comparison of observed variation with that expected as a result of natural selection. Analyses of selection on fitness-related traits by reciprocal introductions served as guidelines for the expected pattern of RAPD variation. We found no concordance between the observed pattern of population genetic structure and that expected under the null hypothesis of environment-specific natural selection. There was no relationship between genetic distance and environmental similarity; none of 54 putative loci exhibited an allele distribution in accordance with that expected and no favoured epistatic allele combinations were detected across the four environments. The fact that environmentally induced adaptation, detected by fitness-related traits, was not reflected in inter-population RAPD structure (1) strongly enhances the neutralist viewpoint and (2) casts doubt on the notion that significant correlations between some environmental parameters and allele frequencies in one or more loci are evidence of selection on the latter.

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### INTRODUCTION

Molecular (allozyme and DNA) variation in plants at inter- and intra-population levels is well documented (references in Ennos, 1983; Hedrick, 1986; Levin, 1988; Linhart & Grant, 1996). Observed genetic differentiation has been interpreted as either the result of localized selection processes (Hamrick & Allard, 1972; Zangerl & Bazzaz, 1984; Lack & Kay, 1988; Van Rossum *et al.*, 1997; Owuor *et al.*, 1999; Li *et al.*, 1999)

or due to local seed/pollen dispersal (Schaal, 1975; Waser, 1987). The association of genetic variation with discrete environmental heterogeneity was considered a criterion for assigning population substructure as being due to natural selection. Absence of such association or failure to detect relevant environmental homogeneity were usually taken as evidence of local gene dispersal. Simulation studies showed that population substructure develops rapidly under isolation-by-distance models without spatially heterogeneous selection (Turner, Stephens & Anderson, 1982; Sokal & Wartenburg, 1983). The interaction of selection with local gene dispersal can either reinforce or retard population differentiation, depending on whether polymorphism is independent of locality or not. When fitnesses of alleles of polymorphic loci are locality dependent, limited gene flow may enhance population differentiation (Maynard Smith, 1966; Dickinson &

\* Corresponding author. E-mail: volis@bgumail.bgu.ac.il

† Current address: The Albert Katz Division of Dryland Biotechnologies, The Jacob Blaustein Institute for Desert Research, Ben-Gurion University of the Negev, Sede Boqer 84990, Israel.

‡ Current address: Department of Nature Conservation, University of Stellenbosch, P. Bag X01, Matieland 7602, South Africa.

Antonovics, 1973), while selection that is locality independent will retard it (Epperson, 1990). There are logistic limitations to the detection of spatially variable selection through direct tests of local adaptation. Consequently, the number of tested habitats/locations is usually small. To date, large-scale studies of genetic structure in natural plant populations using molecular markers (allozymes and DNA) were unable to segregate stochastic and deterministic causes of population differentiation. While in many studies the effect of natural selection on allozyme (Heywood & Levin, 1985; Lack & Kay, 1988; Mopper *et al.*, 1991; Lönn, 1993; Van Rossum, 1997; Lönn, Prentice & Bengtsson, 1996) and DNA (Dawson *et al.*, 1993; Latta & Mitton, 1997; Owuor *et al.*, 1997; Pakniyat *et al.*, 1997; Li *et al.*, 1999, 2000a, b; Johnston *et al.*, 2001) population genetic structures was suggested, a causal relationship between observed pattern of variation and acting selective force(s) has never been demonstrated.

We tested for the presence/absence of spatially variable selection effects on DNA markers starting with the null hypothesis that strong environment-specific (=region specific) natural selection is operating directly (or indirectly through hitchhiking) on molecular markers. We considered such an inversion of a conventional null hypothesis (no selection) and an alternative hypothesis (selection) an essential step in our analysis for several reasons:

First, proving of selection effect on either a multi-locus combination or individual locus is impossible without direct test of individual fitness or analysis of progeny. However, disproving the effect of selection on a particular loci set is possible via a comparison of expected and observed patterns of variation. If there is two patterns' congruence the null hypothesis will hold, but in a case of discrepancy it will be rejected. The second reason for the inversion of the null and alternative hypotheses is that the interpretation of molecular marker variation in natural plant populations as being due to selection (despite its largely speculative nature) became a truism in many studies (see reviews of Hedrick, Ginevan & Ewing, 1976; Nevo, 1983; Hedrick, 1986; Singh *et al.*, 1999). As pointed out by Hedrick *et al.* (1976), when populations are sampled over space there is always a clinal pattern in one or more loci that correlates with some environmental parameter, e.g. rainfall, temperature or salinity. Although this may indicate a selection effect, no such conclusion can be made without appropriate tests of individual fitness under different environmental regimes. Therefore, we designed an experiment where environment-specific selection was explicit and detected de facto by reciprocal transplant introduction of seeds and seedlings (Volis *et al.*, unpublished data). Population genetic structure estimated by RAPD putative loci had to be compared with

that expected under environment-specific selection. In the case of significant discrepancy between observed and expected genetic variation patterns, the null hypothesis can be rejected.

We sampled 20 populations of wild barley, *Hordeum spontaneum*, in four distinct environments of Israel, five within each environment (group), and estimated the genetic relationships of the populations. As populations within each group were sampled in identical or near identical environments and population groups represented environments chosen a priori, strong selection should lead to high similarity of populations within a group. The null hypothesis that natural selection caused population differentiation can be rejected if there is no difference in the similarity of populations from the same and different groups. However, higher similarity of populations from the same groups could be due to natural selection, local gene dispersal, or both. In such a case the next step would be identification of genotypes and comparison of groups. Under natural selection, groups that are spatially distant but environmentally similar are expected to be genetically more similar than groups that are distant both spatially and environmentally. The probability of having the same genotypes favoured by identical selection forces should be higher in environmentally similar groups. Ohta's two-locus linkage disequilibrium analysis of population subdivision (Ohta, 1982) may efficiently detect an effect of epistatic natural selection, if conducted for each environment separately. Ohta's model predicts increasing differentiation of gamete types among subpopulations (correlation of non-allelic genes within a subpopulation) under limited migration as a consequence of random genetic drift. In contrast, if epistatic natural selection is responsible for linkage disequilibrium but not for local differentiation through genetic drift, gametes with favourable combinations of alleles will be selected for in every subpopulation. The ratios  $D_{ST}^2/D_{IS}^2$  and  $D_{1S}^2/D_{ST}^2$  are measures for testing which of two factors (epistasis or limited migration) is responsible for observed linkage disequilibrium. Spatial and temporal heterogeneity of environments occupied by natural populations precluded wide applicability of Ohta's test, because of possible local selection. In our study, with environments chosen a priori and sampling within each environment in near-identical habitats, Ohta's model is appropriate. According to the null hypothesis of environment-specific selection, the same genotypes must be favoured in populations comprising a group, otherwise local differentiation through genetic drift will evolve.

The ability of RAPDs to sample from different regions of the genome and thus to examine multiple loci makes them (under certain limitations) (Hadrys *et al.*, 1992; Lynch & Milligan, 1994) suitable markers for a

test of natural selection on population genetic structure. Although some authors consider the RAPD diversity as near-neutral and maintained by a mutation-drift balance because RAPDs represent the loci mainly in non-coding regions (Williams *et al.*, 1990), there is a considerable number of studies where the observed RAPD variation is interpreted as due to natural selection (Nevo *et al.*, 1996; Latta & Mitton, 1997; Fahima *et al.*, 1999; Cooper, 2000), and in particular studies in wild barley (Dawson *et al.*, 1993; Baum *et al.*, 1997; Owuor *et al.*, 1997, 1999; Nevo *et al.*, 1998). As putative RAPD loci can either be uniformly affected by stochastic factors, if they are selectively neutral, or show different patterns of variation depending on the intensity of natural selection on a particular locus, genetic similarity between populations and population groups must be estimated both across all loci and for each locus separately.

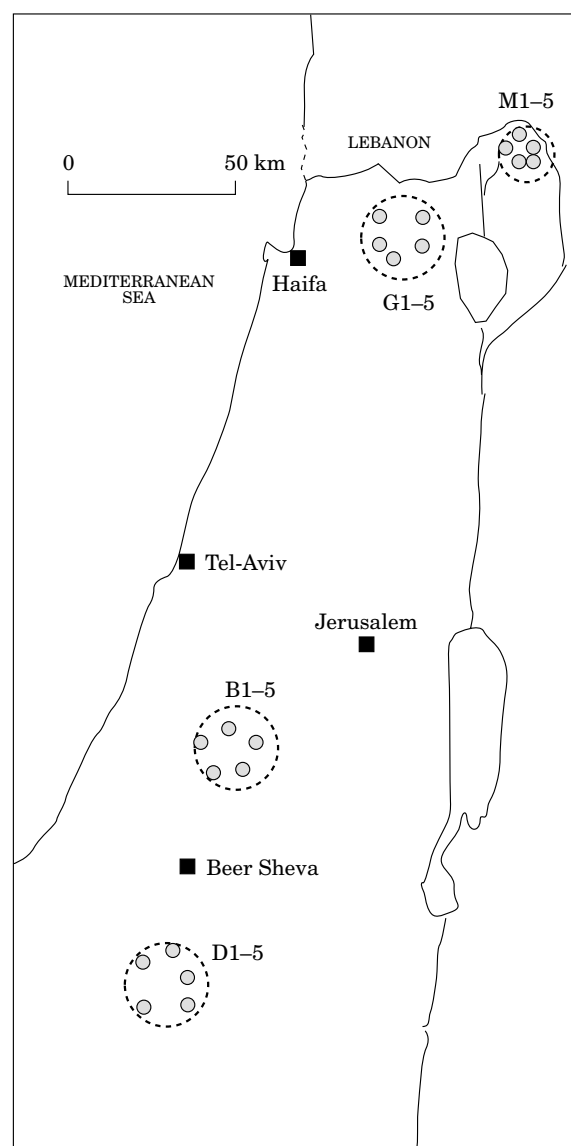
## MATERIAL AND METHODS

### PLANT MATERIAL

The plant material used in this study consisted of 20 populations sampled in Israel in 1996 employing a nested sampling design. Each of five populations, collected in environments that were as similar as possible, comprised a group representative for one of the following environments/vegetative communities: desert, semi-steppe batha, Mediterranean grassland and mountain. Relief, slope exposition, vegetation and soil types were kept constant in sampling localities within a group. Each group can be depicted as a circle of 5 km (mountain) or 20 km (other three groups) in diameter (Fig. 1). All 20 populations were used in a study of population genetic structure, and one (pivot) population from each group was used in a comparative study of plant life histories and in a test of local adaptation (Volis *et al.*, 2001 submitted, manuscript available upon request). Significant genotype-by-environment interactions and highest fitness of genotypes in indigenous environments (except batha genotypes that had highest fitness in the grassland transplant site) were found and indicated local adaptation.

### DNA EXTRACTION AND PCR AMPLIFICATION

RAPD analysis was performed using DNA from 14 plants per population. DNA was purified from fresh 10-day-old leaves (50 µg) ground to a powder in microfuge tubes under liquid nitrogen. For extraction, we used the modified method of Edwards, Johnstone & Thompson (1991), based on addition of purification steps. Re-precipitation of the DNA preparation with 5 M potassium acetate following the phenol extraction was necessary to obtain reproducible PCR profiles.



**Figure 1.** Geographic distribution of sampling localities of wild barley in Israel: M1-5, mountain; G1-5, grassland; B1-5, semi-steppe batha; D1-5, desert.

Each amplification was performed in a reaction volume of 25 µl containing 20 mM Tris-HCl (pH 8.0), 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 200 µM dNTPs, 5 pmols of the primer, 0.5 units of *Taq* DNA polymerase and 25 ng of wild barley DNA. The nine primers of 10 bp in length (Table 1) were selected from Operon Technologies kits A, B, C and F and from 10 primers from University of British Columbia. The main criterion for selection was production of clear amplified polymorphic bands that were replicable in two test reactions.

The RAPD reactions were carried out in MJR PTC-100 Thermo Cycler under the following conditions: 94°C for 3 min, followed by 45 cycles of 94°C for 1 min,

**Table 1.** Primers used in the RAPD analysis, their sequence, number of bands and size range. 'OP' and 'UBC' series are primers from Operon Technologies Inc. and University of British Columbia, respectively

Primer	Sequence	Number of scorable bands	Size range of scorable bands
OPA-17	5'-CCC GCC GTT G-3'	8	670–1750
OPB-05	5'-TGC GCC CTT C-3'	6	850–1300
OPC-05	5'-GAT GAC CGC C-3'	1	870
OPC-09	5'-CTC ACC GTC C-3'	5	1100–2000
OPC-15	5'-GAC GGA TCA G-3'	8	700–2200
OPF-07	5'-CCG ATA TCC C-3'	6	450–1550
OPF-11	5'-TTG GTA CCC C-3'	3	650–1250
OPF-12	5'-ACG GTA CCA G-3'	11	480–1900
UBC-475	5'-CCA GCG TAT T-3'	6	210–440
Total		54	

35°C for 1 min, 72°C for 2 min and final extension 72°C for 7 min. Amplified DNA was immediately analysed or stored at –20°C. The amplification products were analysed by electrophoresis in 1.4% TBE agarose gel (Hispanagar, Spain), stained with ethidium bromide and photographed under UV light. The molecular weight of all bands was calculated by comparison with the  $\phi$ X174/HaeIII marker run in three lanes. All reactions were repeated at least twice and only reproducible bands were scored for statistical analysis. Figure 2 shows an example of RAPD pattern using the UBC475 primer. Only the bands at 440, 350, 300, 260, 240 and 210 bp were scored from this gel. Other visible bands were not considered because of their ambiguous nature.

#### DATA ANALYSIS

Bands were scored as present (1) or absent (0) by independent observers using Tina 2.10g software (Renium, Jerusalem). Bands of identical size amplified with the same primer were considered to be the same locus consisting of two alleles. This interpretation could be justified because barley exhibits very high rate of selfing (99%) (Brown *et al.*, 1978). As they are selfers, the widely recorded problem of RAPDs being dominant markers are not of relevance in this study. A set combining all loci, and three sets from three loci each were analysed. A criterion for choosing these three sets of loci was an agreement of allele distribution among four regional gene pools with three possible patterns expected under environment-specific selection.

Similarity among populations was computed as Nei's (1972) index of genetic distance from the population allele frequencies. Cluster and principal coordinate analyses were performed by NTSYSpc version 2.0

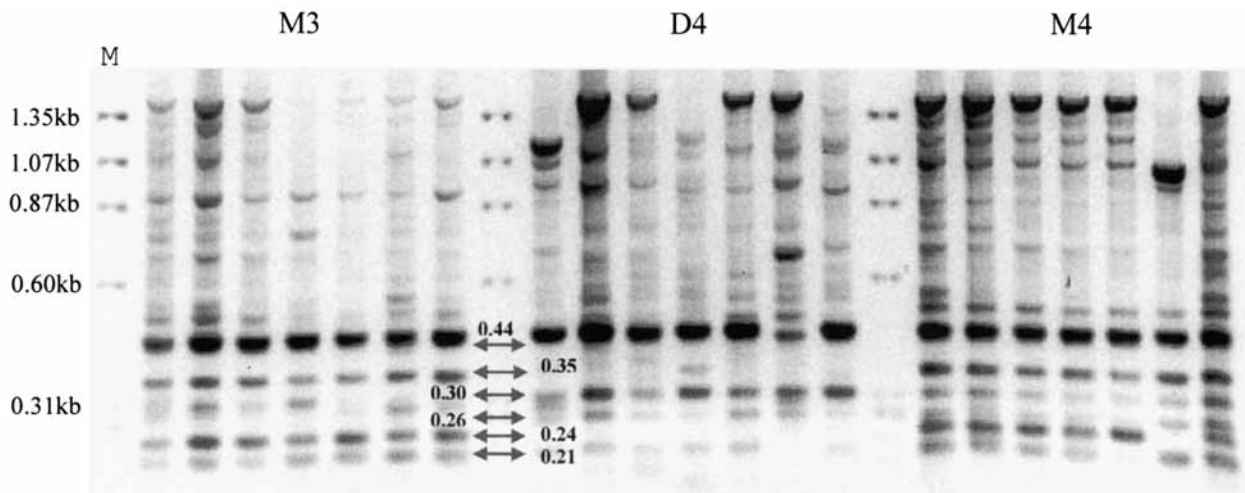
(Rohlf, 1998). Other analyses were done with POPGENE version 1.31 (Yeh, Rong-Cai & Boyle, 1998).

The partitioning of genetic diversity into its three components, within populations, between populations of a group and among groups, was accomplished by using Nei's  $G_{ST}$  statistic (Nei, 1973). The average level of gene flow among populations within a group was estimated from  $G_{ST}$  as approaching  $F_{ST}$  (Slatkin & Barton, 1989).

We performed Ohta's (1982) two-locus population subdivision analysis ( $D$ -statistics). In this analysis, the variance in linkage disequilibrium is partitioned into the following components:  $D^2_{IS}$  (variance of within-subpopulation disequilibrium),  $D^2_{ST}$  (variance of correlation of genes of the two loci of different gametes of one subpopulation relative to that of the total population),  $D^2_{IS}$  (variance of the correlation of genes of the two loci of one gamete in a subpopulation relative to that of the total population),  $D^2_{ST}$  (variance of the disequilibrium of the total population), and  $D^2_{IT}$  (total variance of disequilibrium).

The Ewens–Watterson test for neutrality (1000 permutations per test) (Manly, 1985) was performed on regional gene frequencies considering individuals of five populations as a single gene pool to detect the possible effects of selection on inter-regional allele distribution. The test was run for each locus.

To compare the similarities of populations from the same groups with similarities of populations from different groups, Nei's (1972) genetic identities were calculated: (1) for all pairs of populations within a group; and (2) for five populations of a group with all other populations. Mean genetic identities based on 10 pairwise comparisons ( $I_{within}$ ) and 75 pairwise comparisons ( $I_{among}$ ) were compared for each population group by bootstrapping with random reassignment to regenerate the original unequal sample sizes. This was



**Figure 2.** RAPD amplification products generated from *Hordeum spontaneum* genomic DNA from seven individuals representing M3, D4 and M4 populations obtained with primer *UBC-475*. M— $\Phi$ X174/HaeIII Marker.

**Table 2.** Ecogeographic data for four environments sampled in present study

Environment	Region	Soil	Altitude (m)	Climatic parameters					
				Hu	Rn	Rd	Tm	Ta	Tj
Mountain	Mount Hermon	Terra Rossa	1500	52	1600	70	11	20	1
Grassland	Upper Galilee	Terra Rossa	300	48	580	50	19	26	10
Semi-steppe batha	Shefela Hills	Rendzina	270	47	408	37	19	26	11
Desert	Negev Desert	Loess	470	36	90	15	19	26	9

Abbreviations: Hu—humidity at 14:00, Rn—annual rainfall (mm), Rd—number of rainy days, Tm—annual temperature, Ta—temperature in August, Tj—temperature in January.

repeated 1000 times using the program RESAMPLING STATS (Simon, 1995).

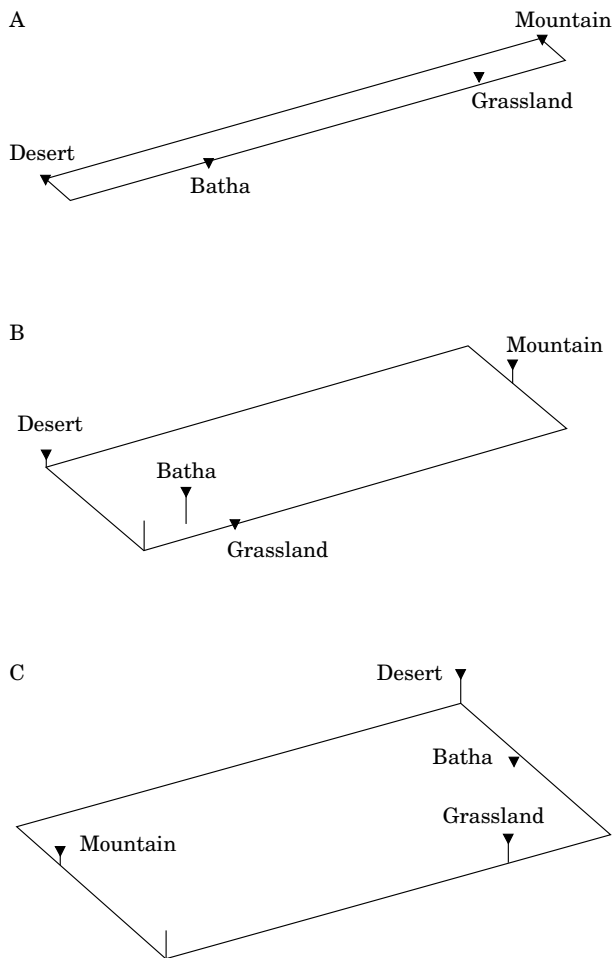
## RESULTS

### GENETIC SIMILARITY

Similarity between the four environments was assessed by principal coordinate analysis (PCA) performed on six climatic parameters and one geographic parameter (altitude) (Table 2). The most similar were batha and grassland, and both environmental extremes (desert and mountain) were clearly distinct from other environments. However, batha and grassland are geographically closer to different environmental extremes, viz. batha to desert and grassland to mountain (Fig. 3A, B).

Genetic similarity between the four group gene pools (pooled allele frequencies in 54 putative loci over five

populations comprising a group) was calculated from the group allele frequencies presented in Table 3. There was high desert-batha and batha-grassland similarity, while desert-grassland similarity was low. Mountain gene pool was very dissimilar from other three groups (Fig. 3C). The matrix of genetic distances was significantly correlated with the geographic distances between the four population groups (but not with the matrix of environmental similarities) (Table 4). Within all four population groups, the populations were genetically more similar to one other than to populations from other groups. On average, pairwise Nei's genetic identities calculated for populations comprising a group were significantly higher than those calculated for populations from different groups (Table 5). Because the overall similarity between population groups may obscure the effects of genetic drift and/or selection on particular loci, we performed the Ewens-Watterson



**Figure 3.** Three-dimensional representation of principal coordinate analysis of geographic distances between four environments (A); climatic similarities between four environments (B); and Nei's genetic distances between four regional gene pools (C).

test for neutrality for each locus (Table 3). In only two putative loci out of 54 (*UBC475-2* and *UBC475-3*) did the pattern of inter-group allele frequencies have significant probability of occurring by chance (<5%) and in another two (*OPC15-3* and *OPF12-1*) it was marginally significant (5%). The allele frequencies in population groups at other 50 putative loci could be explained by chance alone.

Comparison of populations over all 54 putative loci by cluster analysis (CA) (Fig. 4) and PCA (Fig. 5) shows that all five populations from the mountain environment comprise a single cluster that is very distinct from the other 15 populations, and other populations exhibited a trend for clustering consistent with their environments. UPGMA cluster analysis clustered

populations from desert and batha together and grassland and mountain populations separately and distinctly. The three principal coordinates separated all four population groups in three-dimensional space.

To test for a possible effect of environment-specific natural selection on particular putative loci, we identified three sets of loci whose presence/absence allele frequencies in four regional gene pools were most congruent with three possible patterns expected under environment-specific selection. These scenarios were: (a) batha and grassland genotypes are distinct because of selection for high vigour and competitiveness in productive and predictable environments; (b) desert genotypes are distinct due to adaptation to drought tolerance/resistance; and (c) mountain genotypes are distinct due to adaptation to frost tolerance/resistance. The loci that entered the corresponding sets had distinct absence or presence allele frequencies in environment(s) predicted by the corresponding scenario. The batha/grassland set consisted of *OPA17-3*, *OPC9-1*, *OPF11-2*; the desert set of *OPC9-3*, *OPF12-1*, *OPF12-9*; and the mountain set of *OPA17-1*, *OPF12-11*, *UBC475-5*. For example, for the locus *OPA17-3* (batha/grassland set) the frequency of presence allele was 0.086 and 0 for batha and grassland, while it was 0.457 and 0.514 for desert and mountain, respectively. In the locus *OPC9-3* (desert set) the frequency of presence allele was 0.614 for desert and 0.229, 0.057, 0.014 for batha, grassland and mountain, respectively. In contrast, in the locus *OPA17-1* representative for mountain set, presence allele had frequency 0.129 for mountain and 0.986, 0.986, 0.989 for desert, batha and grassland, respectively. We tested the congruence of inter-population allele frequency distributions with each of the above three scenarios under environment-specific selection using both CA and PCA. The results of two analyses were very similar (Figs 6, 7). None of three separate CA and PCAs conducted on three loci sets segregated the populations in a manner congruent with that expected under selection, although the mountain populations were distinct from the others. However, there was also a high dissimilarity of mountain populations from one another (Figs 6C, 7C), which is contrary to the pattern expected under environment-specific selection. Strong environment-specific selection (frost in the mountains) should result in similar allele frequencies across all five populations. In two other loci sets, the discrepancy between expected and observed patterns of inter-population allele frequency distributions was obvious: only three of five desert populations (Fig. 6B) were clustered together and no segregation was found for batha/grassland populations (Fig. 6A). Similar results were furnished by the PCAs (Fig. 7). The population allele frequencies for each of nine loci comprising corresponding sets can be seen on Figure 8. Only two loci *OPA17-1* and *UBC475-5* (both

**Table 3.** Partitioning of the total genetic diversity ( $H_C$  is within population component,  $G_{CS}$  is proportion of inter-population differentiation within a region and  $G_{ST}$  is proportion of inter-region differentiation), allele frequencies in four population groups, and results of the Ewens-Watterson test performed on population groups' gene pools for each locus. Sample size was 280 haplotypes for all loci and groups. Significantly low homozygosities are in bold type

Locus/allele	Molecular weight	Variation component			Population group				Homozygosity F	Corresponding percentage point*	
		$H_T$	$H_C$	$G_{CS}$	$G_{ST}$	Desert	Batha	Grassland			Mountain
<i>OPA17-1</i>	1750	0.37	0.08	0.21	0.72	0.986	0.986	0.929	0.129	0.63	20
<i>OPA17-2</i>	1600	0.30	0.16	0.37	0.12	—	0.100	0.329	0.286	0.71	30
<i>OPA17-3</i>	1500	0.39	0.16	0.45	0.26	0.457	0.086	—	0.514	0.61	20
<i>OPA17-4</i>	1410	0.29	0.19	0.22	0.15	0.414	0.129	0.157	0.014	0.71	30
<i>OPA17-5</i>	1140	0.19	0.12	0.24	0.11	0.729	0.971	0.900	0.986	0.81	35
<i>OPA17-6</i>	900	0.02	0.02	0.18	0.03	—	0.43	—	—	0.98	80
<i>OPA17-7</i>	700	0.13	0.10	0.07	0.15	0.43	0.243	—	—	0.87	45
<i>OPA17-8</i>	670	0.15	0.12	0.06	0.14	0.929	0.757	1.00	1.00	0.85	40
<i>OPB5-1</i>	1300	0.07	0.06	0.12	0.02	0.943	0.943	1.00	0.971	0.93	60
<i>OPB5-2</i>	1200	0.44	0.25	0.31	0.18	0.786	0.500	0.471	0.943	0.56	15
<i>OPB5-3</i>	1000	0.25	0.13	0.32	0.21	0.043	0.129	0.414	—	0.75	30
<i>OPB5-4</i>	950	0.10	0.09	0.09	0.06	0.086	0.129	—	—	0.90	50
<i>OPB5-5</i>	900	0.02	0.02	0.10	0.01	1.00	0.986	1.00	0.971	0.98	80
<i>OPB5-6</i>	850	0.04	0.03	0.16	0.04	0.14	—	0.071	—	0.96	70
<i>OPC5-1</i>	870	0.46	0.12	0.45	0.50	0.029	—	0.686	0.700	0.54	10
<i>OPC9-1</i>	2000	0.46	0.24	0.33	0.22	0.414	0.829	0.900	0.429	0.54	10
<i>OPC9-2</i>	1800	0.20	0.13	0.24	0.15	0.929	0.957	0.986	0.671	0.80	35
<i>OPC9-3</i>	1450	0.35	0.15	0.40	0.32	0.614	0.229	0.057	0.014	0.64	20
<i>OPC9-4</i>	1200	0.37	0.19	0.37	0.18	0.829	0.657	1.00	0.514	0.62	20
<i>OPC9-5</i>	1100	0.32	0.15	0.34	0.29	0.943	0.843	0.971	0.429	0.68	25
<i>OPC15-1</i>	2200	0.22	0.06	0.70	0.14	—	0.14	0.214	0.286	0.78	35
<i>OPC15-2</i>	1650	0.29	0.20	0.24	0.10	0.857	0.629	0.829	0.971	0.71	30
<i>OPC15-3</i>	1300	0.49	0.35	0.25	0.07	0.600	0.743	0.443	0.429	0.51	5
<i>OPC15-4</i>	1070	0.19	0.10	0.43	0.02	0.043	0.100	0.100	0.171	0.81	35
<i>OPC15-5</i>	1030	0.45	0.29	0.26	0.11	0.171	0.214	0.457	0.557	0.54	10
<i>OPC15-6</i>	960	0.02	0.02	0.05	0.01	1.00	0.986	1.00	0.971	0.98	80
<i>OPC15-7</i>	880	0.45	0.14	0.36	0.50	—	0.086	0.857	0.414	0.55	10
<i>OPC15-8</i>	700	0.36	0.17	0.44	0.13	0.071	0.157	0.214	0.486	0.64	20
<i>OPF7-1</i>	1550	0.09	0.04	0.49	0.16	—	—	0.200	—	0.90	50
<i>OPF7-2</i>	1300	0.03	0.03	0.09	0.02	0.971	0.957	1.00	1.00	0.96	70
<i>OPF7-3</i>	1200	0.20	0.14	0.24	0.10	0.957	0.843	0.743	1.00	0.80	35
<i>OPF7-4</i>	860	0.29	0.12	0.40	0.32	1.00	1.00	0.814	0.471	0.71	30
<i>OPF7-5</i>	660	0.30	0.09	0.70	0.02	0.086	0.229	0.229	0.200	0.70	30
<i>OPF7-6</i>	450	0.03	0.03	0.15	0.02	—	0.029	0.043	—	0.96	70

continued

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Locus/allele	Molecular weight	Variation component				Population group				Homozygosity		Corresponding point*
		$H_T$	$H_C$	$G_{CS}$	$G_{ST}$	Desert	Batha	Grassland	Mountain	F		
<i>OPF11-1</i>	1250	0.03	0.02	0.31	0.05	1.00	1.00	1.00	0.929	0.96	70	
<i>OPF11-2</i>	850	0.45	0.22	0.34	0.26	0.443	0.71	0.171	0.700	0.55	10	
<i>OPF11-3</i>	650	0.07	0.06	0.13	0.01	0.957	0.943	0.971	0.986	0.93	60	
<i>OPF12-1</i>	1900	0.49	0.33	0.30	0.05	0.357	0.614	0.614	0.629	0.51	5	
<i>OPF12-2</i>	1800	0.43	0.23	0.44	0.02	0.329	0.186	0.357	0.371	0.57	15	
<i>OPF12-3</i>	1710	0.16	0.11	0.22	0.10	0.043	0.071	0.243	—	0.84	40	
<i>OPF12-4</i>	1550	0.46	0.26	0.32	0.15	0.800	0.743	0.329	0.714	0.54	10	
<i>OPF12-5</i>	1180	0.38	0.26	0.16	0.17	0.743	0.757	0.486	1.00	0.62	20	
<i>OPF12-6</i>	1070	0.25	0.17	0.23	0.07	0.286	0.129	0.129	0.029	0.75	30	
<i>OPF12-7</i>	950	0.23	0.17	0.22	0.07	0.229	0.214	0.100	—	0.76	30	
<i>OPF12-8</i>	900	0.21	0.10	0.43	0.14	0.271	0.214	—	—	0.79	35	
<i>OPF12-9</i>	870	0.25	0.14	0.19	0.30	0.529	0.971	0.986	0.943	0.75	30	
<i>OPF12-10</i>	520	0.19	0.16	0.13	0.06	0.071	0.229	0.100	0.029	0.81	35	
<i>OPF12-11</i>	480	0.43	0.17	0.32	0.42	0.086	0.071	0.271	0.814	0.57	15	
<i>UBC475-1</i>	440	0.12	0.09	0.23	0.04	0.914	0.957	0.871	1.00	0.88	45	
<i>UBC475-2</i>	350	0.50	0.25	0.27	0.30	<b>0.214</b>	<b>0.429</b>	<b>0.571</b>	<b>0.957</b>	<b>0.50</b>	<b>2.5</b>	
<i>UBC475-3</i>	300	0.50	0.34	0.15	0.19	<b>0.714</b>	<b>0.457</b>	<b>0.643</b>	<b>0.143</b>	<b>0.50</b>	<b>2.5</b>	
<i>UBC475-4</i>	260	0.43	0.23	0.26	0.28	0.714	0.814	0.943	0.286	0.57	15	
<i>UBC475-5</i>	240	0.29	0.05	0.55	0.58	0.029	—	—	0.686	0.70	30	
<i>UBC475-6</i>	210	0.23	0.13	0.37	0.09	0.857	0.971	0.929	0.700	0.76	30	

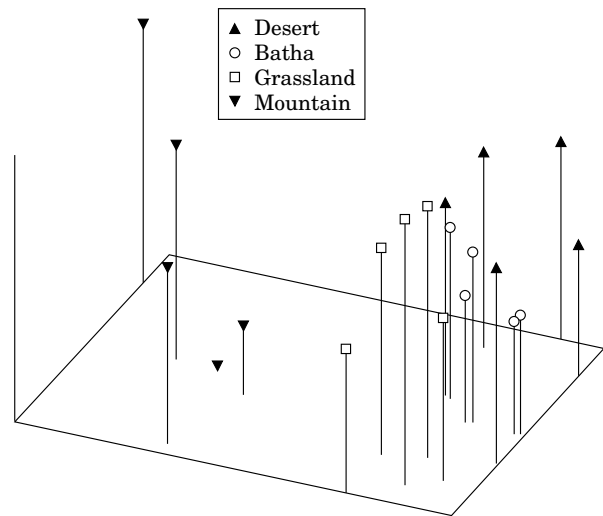
\* Approximate probability of obtaining observed (or lower) homozygosity (Manly, 1985).

**Table 4.** Matrix correlation between (1) environmental similarities (ES) and (2) geographic distances (GD) with Nei's distances calculated for four population groups, tested by Mantel test with 1000 random permutations

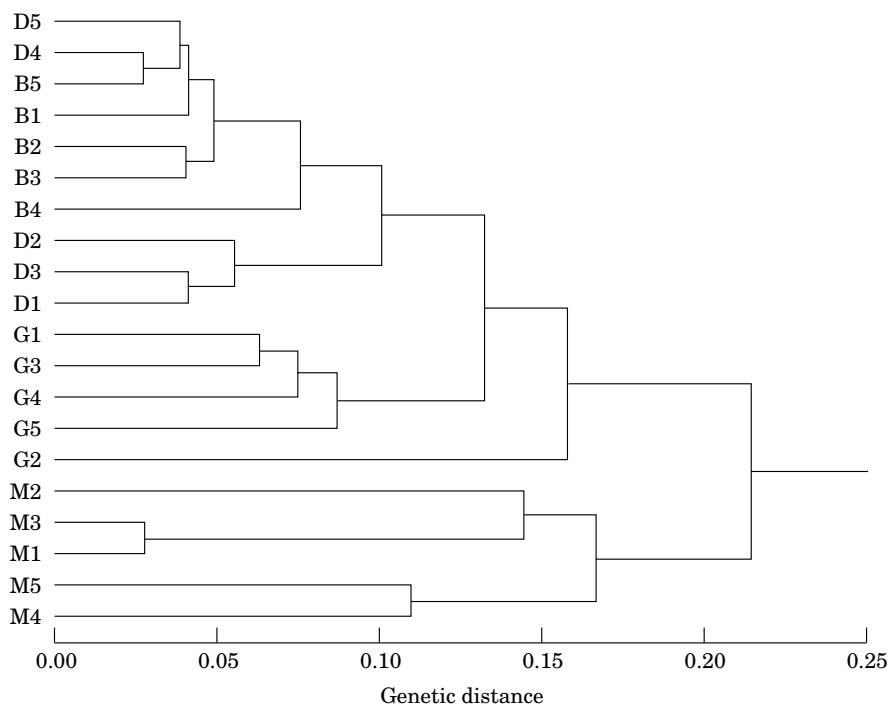
Correlation	<i>r</i>	Mantel <i>t</i> -test	<i>P</i>
ES	-0.80	-1.5	0.210
GD	0.66	2.0	0.046

**Table 5.** Mean genetic identities between populations inside a group ( $I_{within}$ ) and outside a group ( $I_{among}$ ). Number of pairwise comparisons was 10 for  $I_{within}$  and 75 for  $I_{among}$

Group	$I_{within}$	$I_{among}$	<i>P</i>
Desert	0.9317	0.8598	<0.001
Batha	0.9457	0.8732	<0.001
Grassland	0.8976	0.8226	<0.001
Mountain	0.8685	0.8079	<0.001



**Figure 5.** Three-dimensional representation of PCA of Nei's genetic distances between 20 populations of wild barley. Percentage explained variability: PC1, 50%; PC2, 27% and PC3, 14%.



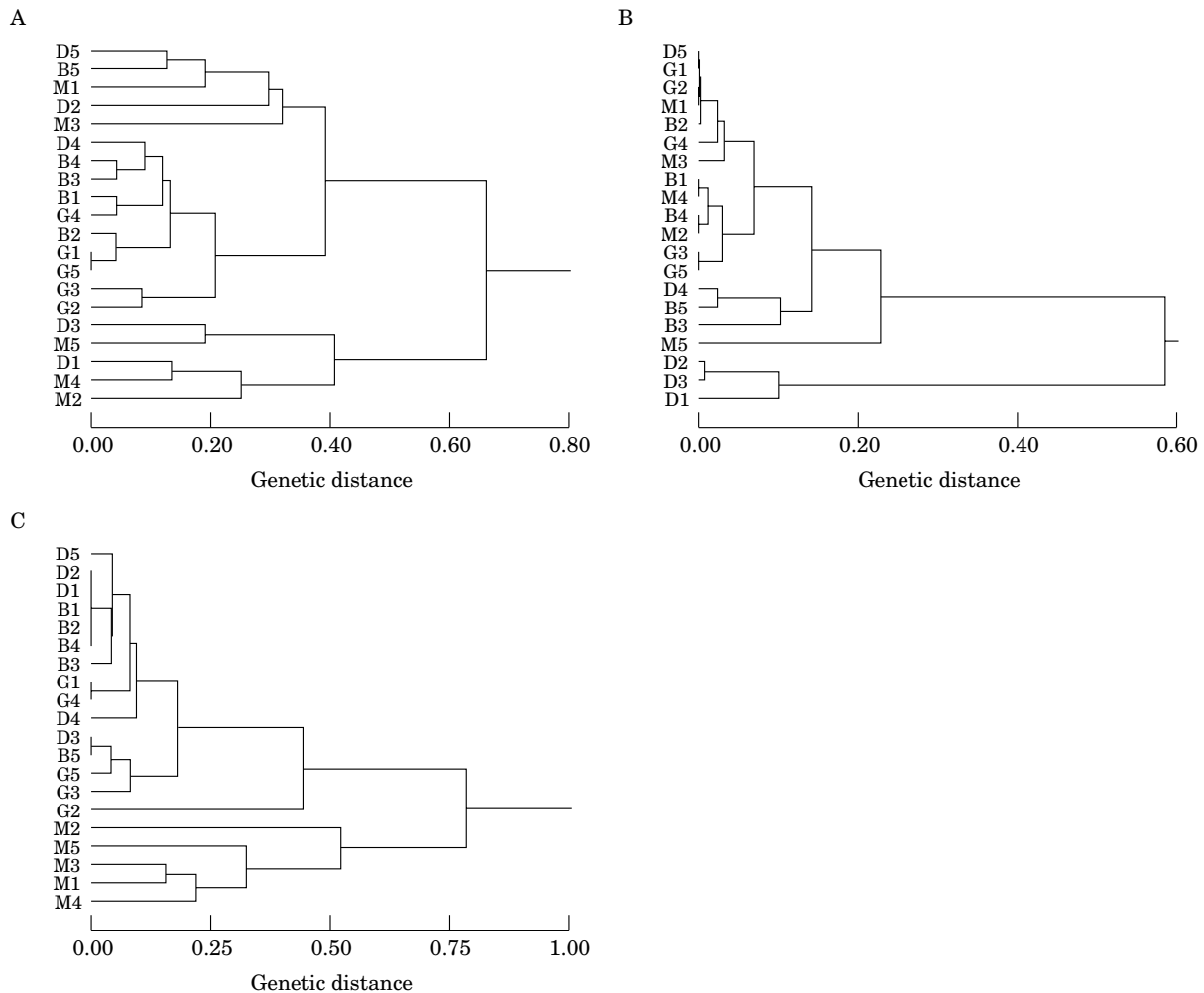
**Figure 4.** UPGMA dendrogram of Nei's genetic distances between 20 populations of wild barley.

from mountain set) exhibited patterns of variation close to expected.

#### GENETIC STRUCTURE

The mean proportion of inter-region (inter-group) variability over all 54 loci was 0.207 while inter-population

variability within a region was 0.311, indicating the importance of both genetic differentiation between population groups and populations within groups (Table 6).  $G_{ST}$  calculated in each population group analysis increased in the following order: batha, desert, grassland and mountain (0.175, 0.231, 0.355 and 0.494, respectively) (Table 6). Gene flow estimated from  $F_{ST}$



**Figure 6.** UPGMA dendrograms of Nei's genetic distances between 20 populations of wild barley obtained from three loci sets. The sets represent loci which frequencies in four regional gene pools are in agreement with three possible patterns expected under environmentally specific selection: (A) batha and grassland are distinct; (B) desert is distinct; and (C) mountain is distinct.

ranged from 2.357 (among desert populations) to 0.512 (among grassland populations). The number of loci with substantial inter-regional  $G_{ST}$  (what we would expect under strong environment-specific selection) was small. Only four loci had  $G_{ST} \geq 50\%$  (*OPA17-1*, *OPC5-1*, *OPC15-7* and *UBC475-5*). Two of these loci (*UBC475-5* and *OPA17-1*) displayed inter-region allele distribution pattern in agreement with the pattern expected under environment-specific selection for frost tolerance/resistance under mountain conditions. However, inter-population variation was also high ( $G_{CS} = 0.55$  and  $0.21$ , respectively).

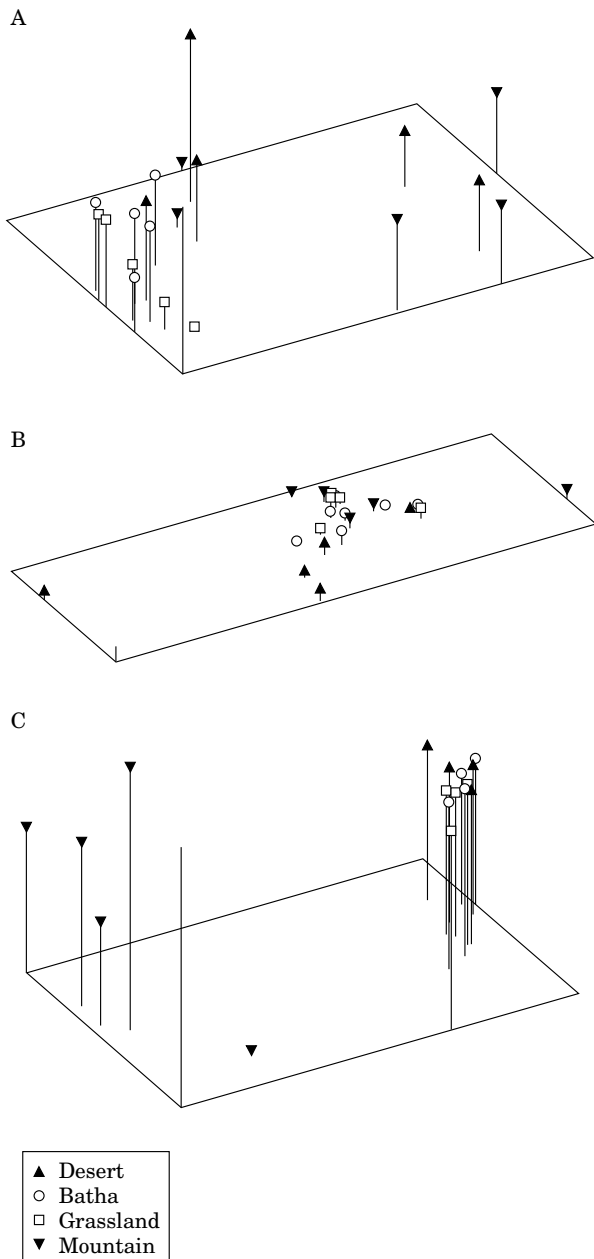
#### LINKAGE DISEQUILIBRIA

Ohta's two-locus analysis of population subdivision performed for each population group revealed consistent relationships for all four groups, viz.  $D_{IS}^2 < D_{ST}^2$

and  $D_{ST}^2 < D_{IS}^2$  (Table 7). This relationship was found for all two-locus combinations, including the loci which inter-region and inter-population allele frequencies were most consistent with those expected under environment-specific selection (*OPA17-1* and *UBC475-5*). This pattern is expected under Ohta's (1982) model for population subdivision under limited migration but not under epistatic natural selection.

#### DISCUSSION

We found that there was greater genetic similarity of populations within the same groups than among populations from different groups. Additionally, the among-group component of variation in allele frequencies approached the among-population variance, indicating the importance of genetic differentiation

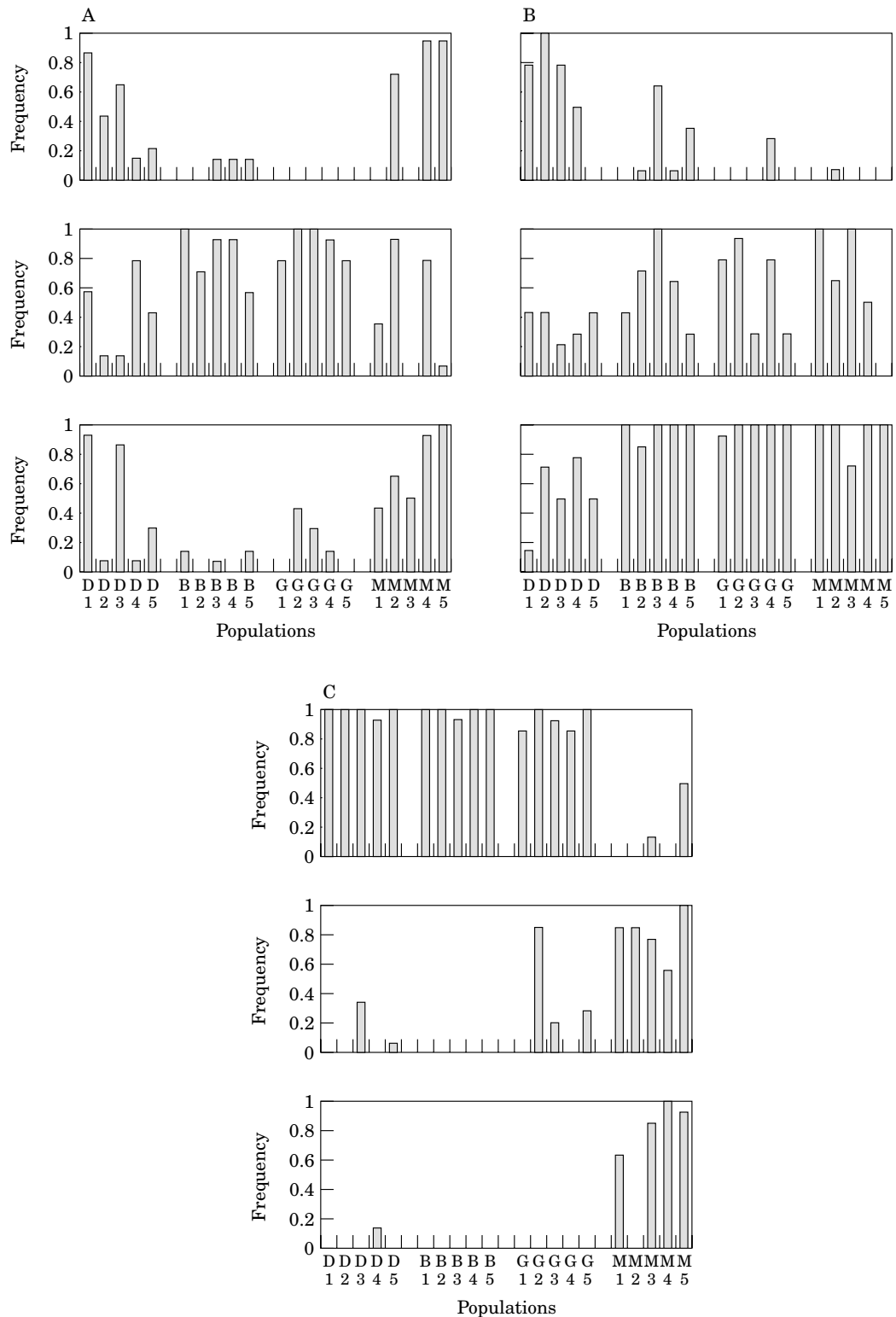


**Figure 7.** Three-dimensional representation of PCA of Nei's genetic distances between 20 populations of wild barley obtained from three loci sets. The sets represent loci which frequencies in four regional gene pools are in highest agreement with three possible patterns expected under environmentally specific selection: (A) batha and grassland are distinct; (B) desert is distinct; and (C) mountain is distinct.

between the four environments. Therefore, our hypothesis of strong environment-specific natural selection can not be rejected on the basis of the overall pattern of population genetic similarities and genetic structure.

A more sophisticated insight into possible consequences of selection in different environments is needed:

- (1) Genetic differentiation between four environments, if caused by natural selection, must be detected at particular loci. The Ewens–Watterson test is an appropriate tool to identify such an effect. At only two out of 54 loci did allele frequencies in the four population groups have a significant probability of occurring by chance (<5%). All other loci were found to be selectively neutral. None of the three sets of loci whose allele distribution among population groups had the highest probability of being due to selection showed the expected pattern of population genetic similarities. None of the expected scenarios (batha and grassland genotypes are distinct because of selection for high vigour and competitiveness in productive and predictable environments; desert genotypes are distinct due to adaptation to drought tolerance/resistance; and mountain genotypes are distinct due to adaptation to frost tolerance/resistance) was fully supported by the results of CA and PCA done on three loci sets.
- (2) If natural selection is environmentally induced, the genetic similarity between population groups must reflect the similarity between their environments and must be independent of geographic distance. Alternatively, if genetic differentiation of population groups is a result of limited gene flow and genetic drift, group similarity should be a function of geographic distance between populations. We found that genetic differences between four population groups do not correlate with environmental differences, but do correlate with geographic distance. However, this is not sufficient to reject the null hypothesis because of small matrix sizes. The observed pattern of inter-group and inter-population genetic similarities seems to reflect a predominant effect of gene flow. High genetic similarity of geographically close and environmentally distant desert and batha populations suggests intensive gene flow with no or a similar effect of selection on group allele frequencies. In contrast, very low genetic similarity of geographically close and environmentally distant populations in grassland and mountain environments is difficult to explain by genetic drift alone. However, here too no locus was detected whose allele frequency distribution could prove its adaptive advantage in the mountain environment. Adequate evidence of such an advantage would be: (1) that the frequency of either presence allele (selected for) or absence allele (selected against) of such a locus approaches zero or one, respectively,



**Figure 8.** Population allele frequencies for three sets of loci (up down): (A) *OPA17-3*, *OPC9-1*, *OPF11-2* (batha and grassland are distinct); (B) *OPC9-3*, *OPF12-1*, *OPF12-9* (desert is distinct); and (C) *OPA17-1*, *OPF12-11*, *UBC475-5* (mountain is distinct).

**Table 6.** Genetic population structure and estimate of gene flow in four population groups. The total variability ( $H_T$ ) is partitioned into either two levels ( $H_S$  is within-population component and  $G_{ST}$  is proportion of inter-population differentiation) or three levels ( $H_C$  is within-population component,  $G_{CS}$  is proportion of inter-population differentiation within a region and  $G_{ST}$  is proportion of inter-region differentiation). Gene flow ( $N_m$ ) is estimated from either  $G_{ST}$  or  $G_{CS}$

Region	Variation component					Gene flow	
	$H_T$	$H_S$	$H_C$	$G_{ST}$	$G_{CS}$	$N_m (G_{ST})$	$N_m (G_{CS})$
Desert	0.200	0.154		0.231		1.667	
Batha	0.207	0.171		0.175		2.352	
Grassland	0.202	0.130		0.355		0.907	
Mountain	0.194	0.098		0.494		0.512	
All regions	0.253	0.201	0.138	0.207	0.311	1.911	1.106
SE	0.003	0.002	0.001				

**Table 7.** Variance components for the observed RAPD LDs (Ohta, 1982)

Groups	Number of pairs of associating alleles	$D^2_{IT}$	$D^2_{IS}$	$D^2_{ST}$	$D'^2_{IS}$	$D'^2_{ST}$
Desert	1865	0.0792	0.0025	0.0763	0.0779	0.0013
Batha	1865	0.0619	0.0026	0.0593	0.0609	0.0010
Grassland	1865	0.1236	0.0023	0.1205	0.1214	0.0022
Mountain	1865	0.1687	0.0025	0.1647	0.1642	0.0045

in populations other than those of mountain origin; and (2) populations from the mountain have similarly high frequencies of alleles selected for or against. The latter is especially true for the mountain as compared with other environments because of the closer proximity of sampled locations (about 1–5 km apart versus 5–20 km for the other three environments). The selection effect on loci comprising the mountain set (*OPA17-1*, *OPF12-11*, *UBC475-5*) is either present together with an effect of genetic drift, or only genetic drift is present.

The last step in our test of the hypothesis that marker variation is adaptive is  $D$ -statistics developed by Ohta (1982) to analyse the causes of non-random associations of alleles: epistatic natural selection and random genetic drift. Ohta's model of linkage disequilibrium in finite subdivided population at equilibrium applied to our data predicts increasing differentiation of gamete types among populations (correlation of non-allelic genes within a population) under limited migration as a consequence of random genetic drift. In contrast, if epistatic natural selection is responsible for linkage disequilibrium but not for local differentiation, gametes with favourable combinations of alleles will be selected for in every population.

Spatial and temporal heterogeneity of environments occupied by natural populations precluded wide applicability of Ohta's test because of possible local selection. In our study, with environmental types chosen a priori and sampling within each environment in near identical habitats, Ohta's model is appropriate. The  $D$ -analysis conducted for each population group separately did not reveal any allele combination that was favoured in a particular environment across all five populations. This means that the observed linkage disequilibrium is due to limited gene flow and genetic drift only, without a noticeable contribution of epistatic selection in each environmental type. The latter is a strong argument for rejecting the hypothesis (adaptive variation in RAPD loci) and to accept the alternative hypothesis (stochastic demographic and environmental causes of variation in RAPD loci in wild barley). The adaptive nature of RAPD markers in wild barley has frequently been mooted (Dawson *et al.*, 1993; Baum *et al.*, 1997; Owuor *et al.*, 1997, 1999; Nevo *et al.*, 1998). Rejection of this hypothesis in our study (which was specifically designed to test this hypothesis) strongly supports the neutralist viewpoint in the neutralist–selectionist debate, and calls into question the concept that significant correlations between environmental parameters and molecular

marker frequencies at one or more loci provide an evidence of selection on the latter (Kojima *et al.*, 1972; Smouse & Kojima, 1972; Johnson & Schaffer, 1973; Clarke, 1975; Nevo, 1983; Nevo & Beiles, 1988). Moreover, our study does not support the hypothesis that seeking differences in differentiation among loci and types of markers is a powerful method for detection of natural selection (Lewontin & Krakauer, 1973; McDonald, 1994; Beaumont & Nichols, 1996). None of the highly divergent patterns of interpopulation variation in RAPD loci exhibited a concordance between the observed pattern of population genetic structure and that expected under the null hypothesis of environment-specific natural selection. Similar results were obtained with allozyme markers (Volis *et al.*, unpublished data) allowing us to question the general use of molecular markers and correlations for inferences about natural selection.

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